to the ground are described in some detail by Sutton (*1*) being attributed to bubbles of overheated air which periodically detach themselves from the thermal boundary layer. In a personal communication, R. W. Stewart, described similar phenomena observed by means of the schlieren method in the cool, unstable thermal boundary of evaporating water. Unsteady thermal conditions in the surface of cooling ponds and sheltered estuaries have been reported by Woodcock and Stommel (*2*).

Further study of the thermal boundary layer should reveal details of the mechanism by which contaminating surface films reduce evaporation from natural bodies of water. In general, it is to be noted that surface temperatures of the ocean or other wind-swept bodies of water determined by conventional methods are systematically biased in a positive sense (*3*).

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New Method for Manipulation, Maintenance, and Cloning of Single Mammalian Cells in vitro

Abstract. Individual mammalian cells can be isolated with the aid of easily handled glass beads to which the cells have become attached. Procedures for the preparation of cell cultures on beads, and for the recognition and manipulation of beads carrying single monocytes, HeLa cells, and Detroit-98 cells are described. Data obtained with *Brucella* infected monocytes, illustrating the efficiency of the method, are presented.

During studies of interactions between *Brucella abortus* strains of different virulence and mammalian monocytes maintained in vitro (*1*), it became apparent that certain problems—specifically, those associated with possible interference phenomena affecting continued ingestion of virulent brucellae—required an analysis of interactions involving single phagocytes. This requirement led to the development of a novel and relatively simple method for isolating single monocytes: exploratory trials indicate that this technique may also be used for isolating other mammalian cell types. The principal feature of the new method is the fixing and manipulation of single cells that have been permitted to adhere to the surface of small glass beads (*2*) partially embedded in a paraffin (or 2 percent agar) layer contained in small glass cups.

Table 1. Distribution of brucellae per monocyte among bead isolates and stained monocytes, determined by plating single monocytes and counting the resultant colonies or by the microscopic observation of stainable intracellular brucellae. Each figure in this tabulation indicates the number of monocytes containing, according to the column in which they are recorded, 0 to more than 10 bacteria.

No. of brucellae per monocyte											
0	1	2	3	4	5	6	7	8	9	10	>10	Total
Plating bead isolates												
11	5	3	6	4	4	2	0	0	1	0	4	40
Stained coverslip No. 1												
12	7	5	4	3	3	2	0	1	0	0	3	40
Stained coverslip No. 2												
9	9	6	6	3	2	1	1	0	1	1	1	40
Stained coverslip No. 3												
10	7	7	5	6	2	0	0	1	0	0	2	40

Before exposure to cell suspensions, the beads are boiled in detergent, washed in distilled water, dried, and sterilized by dry heat. They are then placed in sterile glass cups (5 ml Beckman *p*H-meter cups, cut to a height of approximately 5 mm) containing a thin layer of molten Vaspar (equal volumes mixture of noncarbolated Vaseline and household paraffin). The Vaspar layer is prepared by adding to thoroughly dried, heated cups a small amount of hot (about 100°C) Vaspar, the cups being immediately inverted to remove excess Vaspar. To the liquid Vaspar layer remaining within the cups, beads (about 500 per cup) are added by gentle sprinkling, which results in an even distribution of well-separated beads anchored in the Vaspar layer to about half their depth. Slow cooling of the bead-seeded cups is important and is achieved by placing the cups in heated petri dishes.

To isolate single monocytes, cells are harvested by previously described methods (*3*), and 0.8 ml of an appropriate suspension (containing approximately 5×10^4 monocytes per milliliter in a mixture of autologous serum and Hanks' solution) is added to each cup. The cups are sealed with silicone-greased 1-inch square coverslips, to prevent loss of carbon dioxide and water, and incubated at 37°C. After approximately 2 hours, the cups are examined for the presence of beads with single monocytes on their surface. Such beads may be removed for immediate examination or may be permitted to remain in the cups for any desired period of time.

The recognition of single monocytes on individual beads requires careful microscopic examination using proper lighting. We routinely employed a dissecting microscope with 18× ocular and 6× objective, using a sheet of green blotting paper in place of the usual substage mirror. When properly illuminated, monocytes adhering to the exposed bead surface are visible as differently refracting, irregularly-shaped cells, whereas the air bubbles found in most beads are seen as highly refractive circles.

For removal of the beads from their Vaspar bed, capillary pipettes with a diameter slightly greater than that of the beads were used. Beads were picked up by punching out a single bead plus its Vaspar base, and were removed from the pipette by tapping or by positive pressure.

The efficiency of the method is illustrated by a comparison of the distribution of numbers of brucellae in individual monocytes as determined by the bead method and by direct microscopic examination of stained monocytes (Table 1). For the former procedure, monocytes, infected with brucellae 30 minutes earlier, were introduced into the cups in the presence of streptomycin to kill all extracellular bacteria. Two hours later beads carrying single monocytes were removed and lysed on the surface of nutrient agar with the aid of 2 percent saponin (*4*), and the number of intracellular bacteria was determined by counting the colonies that developed after 5 days. For the procedure employing direct examination, infected monocytes were allowed to adhere to flying-coverslips in the presence of streptomycin, and were stained 2 hours later by Machiavello's technique (*5*). The data in Table 1 indicate a close correlation between the results obtained by these two procedures.

The method of isolating single cells

with the aid of beads has also been applied successfully to manipulation and cloning of other mammalian cell types, namely HeLa carcinoma cells and Detroit-98 bone-marrow cells. Two methods have been employed: (i) beads carrying single cells were immediately transferred to suitable tissue-culture media, or (ii) beads carrying single cells were permitted to remain in the cups until clones of sufficient size had established themselves on each of these particular beads. The latter procedure made it possible to obtain progeny from known single cells in the presence of other cells, a condition that in exploratory trials has proved favorable for the initiation of clones. For the purpose of recording the location of beads initially carrying only single cells, a photographic recording procedure was employed: first the location of every bead in the cup was mapped by projection onto photosensitive paper; 24 hours after the cups had been inoculated with a trypsinized cell suspension, the positions of all beads carrying single cells were marked on this photographic record. It should be added that provisions for the carbon dioxide tension required during the manipulation and growth of these mammalian cells were made in a simple manner: cups were incubated, unsealed, at 37°C in a CO_2-incubator (or in a closed jar in which CO_2-rich conditions were produced by burning a candle to extinction), and were inspected in a CO_2-rich atmosphere that had been created by CO_2-exhaust from a funnel placed adjacent to the microscope stage.

This procedure should prove useful for the primary isolation, manipulation, and cloning of many different cell types, particularly if initial maintenance of selected cells in the presence of other cells is prerequisite to the successful establishment of a clone (*6*).

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Cytochemical Adenosine Triphosphatase of Vorticellid Myonemes

Abstract. A highly specific adenosine triphosphatase has been found to be localized in the contractile apparatus of vorticellids. It is most prominent in the cilia as well as in the myonemes which course the latitudes and longitudes of the cell and spiral in the flat peristome. The stalk displays more activity in the spasmoneme sheath than in the spasmoneme itself. The spasmoneme canal appears negative. A delicate fiber coils around the outside of the stalk in a helix. All these structures may be seen in the living protozoan by phase-contrast microscopy.

A very rich literature supports the hypothesis, originally presented by A. Szent-Györgyi (*1*), that contractility is dependent upon some interaction of adenosine triphosphate (ATP) and actomyosin. An important aspect of this interaction is the adenosine triphosphatase which contractile protein possesses by virtue of its myosin content (*2*). Extracts made from vertebrate (*2*) and invertebrate muscle (*3*) have this enzymatic property. Of fundamental importance is the fact that similar extracts of cells (*4*) or their isolated motor apparatus, sperm tails, (*5*) also display this enzyme activity.

Recently, direct demonstration of adenosine triphosphatase in intimate association with motor organelle was made for the fibers within sperm flagella by a cytochemical method (*6*). It is the purpose of this report to describe the cytochemical locus of adenosine triphosphatase in myonemes, the motor organelle of vorticellids, and other protozoans.

Vorticella convallaria were used throughout. They were derived from a clone cultured for several years by a previously described method (*7*) with some modifications. A cerophyl extract was substituted for the lettuce in the egg-lettuce medium. It is prepared by boiling 1 mg in 1 ml of distilled water for 5 minutes and filtering while hot into flasks which are then plugged and refrigerated. Equal parts of the egg and cerophyl are mixed to provide the culture medium. Routinely, petri dish bottoms were lined with non-nutrient agar before they were filled with the culture medium. Vorticellids detach from such a surface after 3 to 4 days of incubation at 25°C, thereby making for convenient harvest by light centrifugation after filtering through a No. 25 plankton net. They were then washed three times in sterile culture medium or Chalkey's solution before use in subculturing or in cytochemical study.

For assay, 20-μl aliquots of cell suspensions were placed on lightly albuminized cover slips (No. 0, 22 mm) and frozen directly on a block of Dry Ice. They were then thawed and dried with the aid of a fan. When dry, the cover slips were immediately immersed in substrate medium contained in Columbia staining dishes which had been previously equilibrated at 37 ± 0.2°C for 1 hour.

The substrate medium was essentially that of Padyluka and Herman (*8*). It contained 5 mmole of ATP (*9*), 2.5 mmole of cysteine-HCl, 20 mmole of $CaCl_2$, and 25 mmole of Veronal. The *p*H was adjusted to 9.4 and the solution was filtered before equilibration. In some experiments, 5 mmole of ADP, 12 mmole of muscle adenylic acid, (AMP), beta-glycerophosphoric acid, glucose-1-phosphate (*10*), and fructose-1,6-diphosphate (*11*) replaced ATP as substrate.

All substrates were added as the dry powder, and demineralized water was used throughout. All experiments included parallel preparations incubated in control media which lacked substrate. In addition, vorticellids that had been steamed for 10 minutes before incubation were also tested.

After incubation for 15, 30, 60, 180, or 240 minutes, inorganic phosphorus was visualized as black CoS by 3-minute immersion of cover slips in control medium saturated with $(Ca)_3(PO_4)_2$ by the addition of 0.1*M* Na_2HPO_4. They were then placed in 2-percent $CoCl_2$ for 3 minutes, followed by three washes in distilled water. Both the $CoCl_2$ and distilled water were brought to above *p*H 8 by the addition of 25 mmole of Veronal, as recommended by Danielli (*12*). Following this, cover slips were dipped in 1-percent $(NH_4)_2S$ for 2 minutes, washed

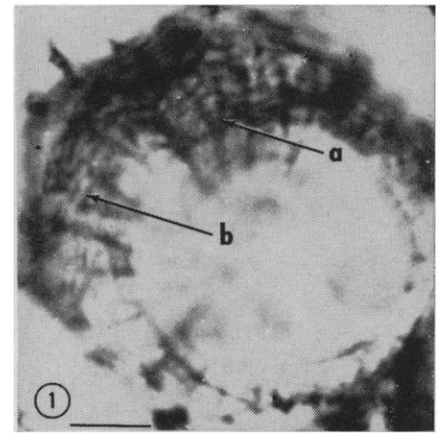

Fig. 1. Sites of adenosine triphosphatase in head. Incubation time, 60 minutes. *a*, Longitudinal myoneme; *b*, latitudinal myoneme. Magnification marker at lower left, 5μ.